Expression of mammalian cytokines by Trypanosoma cruzi indicat unique signal sequence requirements and processing.

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A vector based upon the calmodulin-ubiquitin 2.65 locus of Trypanosoma cruzi ha enabled the expression and secretion of the murine cytokines interleukin-2 (IL-2) a gamma-interferon (gamma-IFN) by transfected T. cruzi. The T. cruzi-derived cytokines were bioactive and produced by both epimastigotes and mammalian forn The native coding sequence of IL-2 was sufficient to cause secretion of the protein the gamma-IFN signal sequence had to be replaced by the IL-2 signal sequence (IL-2/gamma-IFN) to allow efficient secretion of gamma-IFN. The amino acid sequences at the N-termini of the secreted T. cruzi-derived cytokines were differen from the expected murine secreted protein. The secreted IL-2 was cleaved six amir acids downstream from the murine signal sequence cleavage site, and the hybrid IL-2/gamma-IFN molecule was cleaved three amino acids downstream from the predicted signal cleavage site in the IL-2/gamma-IFN molecule. These apparent differences in signal peptide sequence requirements and cleavage sites most likely indicate that the signal sequence processing in trypanosomes is distinct from that o higher eukaryotes.

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1: Behring Inst Mitt 1991 Jul;89:12-22 Related Articles, Bo

Modification of the cleavage activation of the influenza virus hemagglutinin by site-specific mutagenesis.

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Factors determining cleavability of influenza virus hemagglutinin which is activate by ubiquitous cellular endoproteases were analysed by carrying out site-directed mutagenesis on the cloned hemaglutinin genes of strains A/FPV/Rostock/34 (subty H7) and A/Port Chalmers/1/73 (subtype H3). Substitutions at the cleavage site of t H7 hemagglutinin indicate that the tetrapeptide Arg-X-Lys/Arg-Arg is the minimal consensus sequence recognized by the ubiquitous proteases. The H3 hemagglutinir also became susceptible to these enzymes, when additional arginines were inserted the cleavage site. Three arginines were sufficient, when the carbohydrate was removed, whereas four additional arginines are needed when this carbohydrate was present, indicating that the accessibility of the cleavage motif is important for the protease. The appropriate localization of the basic cleavage motif within the amino acid sequence and the spatial structure of the hemagglutinin precursor is an additio prerequisite for cleavage.

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Expression and functional characterization of Escherichia coli Nus and lambda Q as glutathione S-transferase fusion proteins.

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The Escherichia coli transcription factor NusA and the bacteriophage lambda antiterminator Q proteins were expressed as inducible glutathione S-transferase (G fusion proteins. The fusion proteins were purified under nondenaturing conditions affinity chromatography on glutathione agarose. Thrombin cleavage of the glutathi agarose-bound fusion proteins yielded homogeneously pure NusAN+15 (5 mg/g ce and almost homogeneously pure QN+13 protein (0.7 mg/g cells), where N+x indic the presence of x additional amino acids at the N-terminus of the protein. The puri NusAN+15 exhibited the same activities as wildtype NusA in enhancement of transcriptional pausing, enhancement of termination at Rho-independent terminato and enhancement of Q-mediated antitermination in vitro. The QN+13 protein exhibited both anti-pausing and antitermination activities in Q-mediated transcripti antitermination. However, the antitermination activity of QN+13 was lost graduall during storage if the thrombin used for cleavage of the GST fusion protein was not removed. This was due to cleavage by thrombin after Arg22 within the Q protein itself, at a noncanonical thrombin cleavage site, so the truncated protein (QN+22) lacked the first 22 amino acids at the N-terminus of Q. The expression vectors described here can be used to rapidly produce large quantities of these proteins, an the truncated Q protein can be used to evaluate the requirement for the N-terminus Q in antitermination, anti-pausing, interactions with the DNA template (qut site), a interaction with RNA polymerase itself.

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1: Yeast 1995 Nov;11(14):1381-1391

Structural features of a polypeptide carrier promoting secretion of beta-lactamase fusion protein in yeast.

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Escherichia coli beta-lactamase was secreted into the culture medium of Saccharomyces cerevisiae in biologically active form, when fused to the C-terminu the hsp150 delta-carrier. The hsp150 delta-carrier is an N-terminal fragment of the yeast hsp150 protein, having a signal peptide and consisting mostly of a 19 amino peptide repeated 11 times in tandem. Here we expressed the hsp150 delta-carrier fragment alone in S. cerevisiae. Apparently due to a positional effect of the gene insertion, large amounts of the hsp150 delta-carrier were synthesized. About half o the de novo synthesized carrier molecules were secreted into the culture medium, t rest remaining mostly in the pre-Golgi compartment. The extensively O-glycosylat carrier fragment was purified from the culture medium under non-denaturing conditions. Circular dichroism spectroscopy showed that it had no regular seconda structure. Nuclear magnetic resonance spectroscopy showed that a non-glycosylate synthetic peptide, the consensus sequence of the repetitive 19 amino acid peptide, lacked secondary structure. The unstructured carrier polypeptide may facilitate pro folding and secretion of heterologous proteins attached to it.

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